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THE REGULATION OF RENAL ACTIVITY

I. Regulation of Urea Excretion by the Concentration of Urea in the Blood and in the Urine

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Received for publication January 28, 1918

Renal activity expresses itself in the formation of urine from blood. But the urine is a complex product containing many substances which the kidney has separated from the blood. Each of these substances may call for a different type and degree of activity and each will require separate investigation before any comprehensive idea of the regulation of the action of the kidney as a whole can be attained. In the preliminary work on this general problem which is reported here, we have not attempted such an all inclusive study but have confined ourselves to the factors regulating the process of urea excretion. We felt that it would be advantageous to work at first with one particular substance and only to go on to others after we had obtained some experience in the difficulties which might arise and in the methods best adapted to meet them. We chose urea, partly because it is relatively easy to make accurate and numerous measurements of the urea content of the blood and urine, but mainly because we believed that the regulation of renal activity in the excretion of urea is simpler than that of other important substances dealt with by the kidney, such as water and chlorides. The reasons for this belief have been summarized elsewhere (1).

The excretion of urea, as of other urinary constituents is accomplished by the coördinated activity of "living" kidney cells, and from analogy with other biological phenomena it is to be expected that a large number of factors may directly or indirectly coöperate in determining the rate

at which it is excreted and that there may be an intricate play of action and reaction among these factors. This probable complexity and our quite certain ignorance of even the main features of the process of urea excretion, indicate that the initial work must be of a simple qualitative nature. We must be satisfied, as a first step, with trying to find which of the many possible factors are actually operative and which of these are the more important.

With this end in view we have measured the rate of urea excretion in a large number of animals under certain fixed conditions. Having thus established an average rate and its range of variation, we have proceeded to observe the effect of experimental alterations in one after another of the possible factors, the conditions otherwise being kept as far as possible the same.

As originally planned, and up to a certain point carried out, it was intended that normal human individuals should be the subjects of study but problems presented themselves requiring procedures applicable only in animal experiments, and in the following attempts at their elucidation rabbits have been used. This change made it necessary to start the whole work again from the beginning, for the rabbit's average rate of urea excretion and its variability had to be found for the particular conditions chosen as our standard before it was possible to go on to estimate the degree of influence of any particular factor.

An *a priori* consideration of the subject would suggest that the factors determining the rate of urea excretion may be divided into two classes, those associated with the kidney itself and those associated with the blood and urine which form the immediate environment of the kidney cells. In general the first group will comprise limiting factors which are relatively constant—the quantity and quality of the structure of the renal secreting tissue—while the second group will contain factors which are unstable and fluctuating. If the influence on renal function of these two groups of factors could be separated in practice as well as in theory, if it were possible to measure the effect of the amount and quality of the secreting tissue of the kidney alone, this classification would be of fundamental importance for clinical medicine. It would allow of a distinction between those alterations in function which are of essential value in diagnosis and prognosis because they are permanent and determined by fixed anatomical peculiarities in renal structure, and those which are non-essential and transient because they have their origin in the ever changing environment of the kidney cells. Our ultimate object has been to draw such a distinction to at least such a

degree as to render it of practical value. In attempting this we have first studied the factors associated with the immediate surroundings of the kidneys and have tried to eliminate the influence of the factors associated with kidney structure by working with rabbits of not very diverse weights and particularly by using the same animals repeatedly both under standard conditions and under conditions in which one or other of the factors considered was changed.

There is one variable factor, the concentration of urea in the blood, which would be first thought of as likely to be of importance in determining the rate of urea excretion. A comparison of rates measured over periods during which the blood urea concentration varies does in fact show that, as a general rule, the higher the level of the concentration of urea in the blood, the greater is the rate at which urea is excreted. Now we have not found it possible by any uniformity in external conditions to prevent the occurrence of considerable fluctuations in blood urea concentration even in the same animal. The inconstancy of this factor introduces an internal variable into our standard conditions which is beyond our power to control and whose effect is to produce such wide differences in rates measured under these standard conditions that the effect of any other factor experimentally introduced would have to be very marked indeed to be capable of demonstration. But the fact that this variable can be measured and taken into consideration in comparing rates, puts into our hands a means of obviating this difficulty. When we find that rates measured at the same blood urea concentration are nevertheless different, we may conclude that the difference is due to the intervention of other factors than blood urea concentration. Or when we find that by increasing the incidence of any particular factor we sensibly alter the average rate from that obtained at the same levels of blood concentration under our standard conditions, we may regard this alteration as an indication that the factor in question is operative, and we may take the nature and the degree of change which it induces as indicative of its mode of action and of its importance in comparison with other factors.

This first paper, accordingly, deals with the determination of the average rate of urea excretion under uniform external conditions for each level of blood urea concentration. This having been determined it becomes possible to estimate the influence of another variable factor in the environment of the kidney which can be accurately measured, i.e., the effect of variations in the concentration of urea in the urine. These questions have already been dealt with in our work on man, but

apart from the fact that they form a necessary preliminary for further investigation on the rabbit, the observations here recorded extend the scope of the original study so far as the blood urea concentration is concerned, since the effect of a much wider range of variation in concentration could be determined than was possible under the restrictions required by care for the safety and comfort of human subjects.

METHODS

When the animals were not being used they were kept together in large cages in the animal room, except in springtime when it was found necessary to separate them in order to prevent them from killing each other in fights. They were all males. Once a day, about 10 a.m., they were given crushed oats, alfalfa and sometimes stale bread. Water was not restricted. When observations were to be made on any particular animal it was brought to the laboratory on the afternoon of the the previous day and placed in a small metabolism cage. No food and no water was given until the experiment, which began next day at 9 a.m., was ended. Our object was to exclude as far as possible such variations in kidney function as might be associated with differences in the food and water previously taken.

The conditions having thus been made uniform, at least to a certain extent, measures were taken to induce all degrees of variation, including the most extreme, in the concentration of urea both in the blood and in the urine. This was done by the administration of varying quantities of urea and of water.

The time-relationships which, quite unexpectedly, were found to be of considerable importance, were the same in all experiments. Starting in the morning about 9 a.m., the stomach tube was passed in all cases whether a urea solution or water or nothing at all was given. The animal was then placed on its back in a comfortable holder and the bladder emptied by catheter and washed with a known volume of water. It was then returned to a special cage built over a glass funnel so that if by chance any urine were passed between the periods of catheterization, it should not be lost.

Half an hour later a little more than 1 cc. of oxalated blood was obtained by puncturing the marginal ear vein after it had been dilated by warming over an electric light bulb. In the early part of the work, duplicate estimations on 5 cc. quantities were made; later duplicates on 1 cc. amounts, and during the last year with the successive improve-

ments in the technique of aeration and titration which have been described by Barnett (2), we have usually carried out only one estimation on 1 cc. of blood.

One hour after the bladder had been emptied, the urine was collected by catheter, its volume measured and the bladder again washed out with a known volume of water, any excess returned, being added as a correction to the quantity already measured. The mixed urine and wash water was then diluted to an extent dependent on the quantity of urea expected with water containing sufficient H_2SO_4 to make it acid and so prevent decomposition of urea by urease containing organisms. Because they are easier to catheterize only male rabbits were used. Great care was taken to make sure that the bladder was completely emptied. The bladder is often so toneless that simple catheterization is not enough. The abdominal wall must be compressed, and the catheter alternately withdrawn, reintroduced and rotated. There is also a considerable degree of variation in the size of the urethra and the catheter selected must be large enough to prevent urine escaping by its side. A basin or funnel was used to catch urine which, in spite of all precautions, was sometimes passed in this way. In a considerable number of the experiments in which urea was not given, the washing of the bladder was omitted. We were inclined at first to think that the error in the calculation of the urea concentration of the urine which might be introduced by wash water left in the bladder, might be of greater importance than the increased accuracy which this procedure gives to the determination of the rate of urea excretion. In these experiments, however, the volumes of urine were large because of the diuretic effect of the urea, so that the error arising from leaving a little urine in the bladder was relatively small. In experiments in which the volumes of urine were small it was obvious that washing was imperative, and in all these cases it was done.

At the end of the second, third and fifth hours, the same process of catheterization was repeated,and at the middle of each of these periods blood was collected. For each experiment therefore four collections of urine were made, each with its corresponding collection of blood.

The urea estimations in both urine and blood were made with Marshall's urease method, using for the urine the titration method with the modifications we have already detailed, (3) and for the blood the aeration method with the slight changes which we described before, and latterly with the refinements introduced by Barnett (2).

The rate of urea excretion, whether observed over a one or a two hour period, is in all cases given as the rate per hour in milligrams, the blood urea concentration as milligrams per 100 cc. of blood, and urine urea concentration as grams per 100 cc.

The data were collected in this manner because the effect of changes in blood or urine concentration on the rate of urea excretion should be capable of demonstration when the various rates, with the concentrations of urea found to exist during the periods over which they were measured, are compared. In the case of the data on the blood urea concentration this involves the assumption that the concentration measured at the middle of a period of urine collection represents the average concentration existing throughout the whole of that period. This of course is not necessarily the case, but since the periods of urine collection were never more than two hours, the error can seldom have been large and cannot invalidate merely qualitative deductions drawn from the general trend of a large number of observations.

It might also be questioned whether it is right to assume, as we in fact do, that the concentration of urea found in blood removed from an ear vein is the same as that in the blood supplied to the kidney, but since we were unable to find any significant difference in the urea concentration of blood obtained from the jugular vein, the carotid and the femoral artery, and the concentration in blood removed at the same time from the renal artery of the rabbit, we believe that this assumption is justified.

Regulation of urea excretion by the concentration of urea in the blood. In figure 1 each hourly rate of urea excretion is represented as a point on a chart in which the ordinate gives the magnitude of the rate and the abscissa the level of blood urea concentration observed at the time the rate was measured. In these circumstances if there is some relation between the two in the sense, for instance, that an increase in blood concentration is accompanied by an increase in rate, we should find that relation depicted as a rise in the ordinates from the left to the right of the chart. It will be seen that in a general sense there is evidence of such a relationship in the manner in which the points are grouped. The average rate is shown in the curve and demonstrates that, on an average, each increase in blood urea concentration is accompanied by an increase in the rate of urea excretion.

The very pronounced deviations of many of the rates from the curve of the average, make it almost unnecessary to point out that this curve cannot be used as a basis for any mathematical deductions as to the

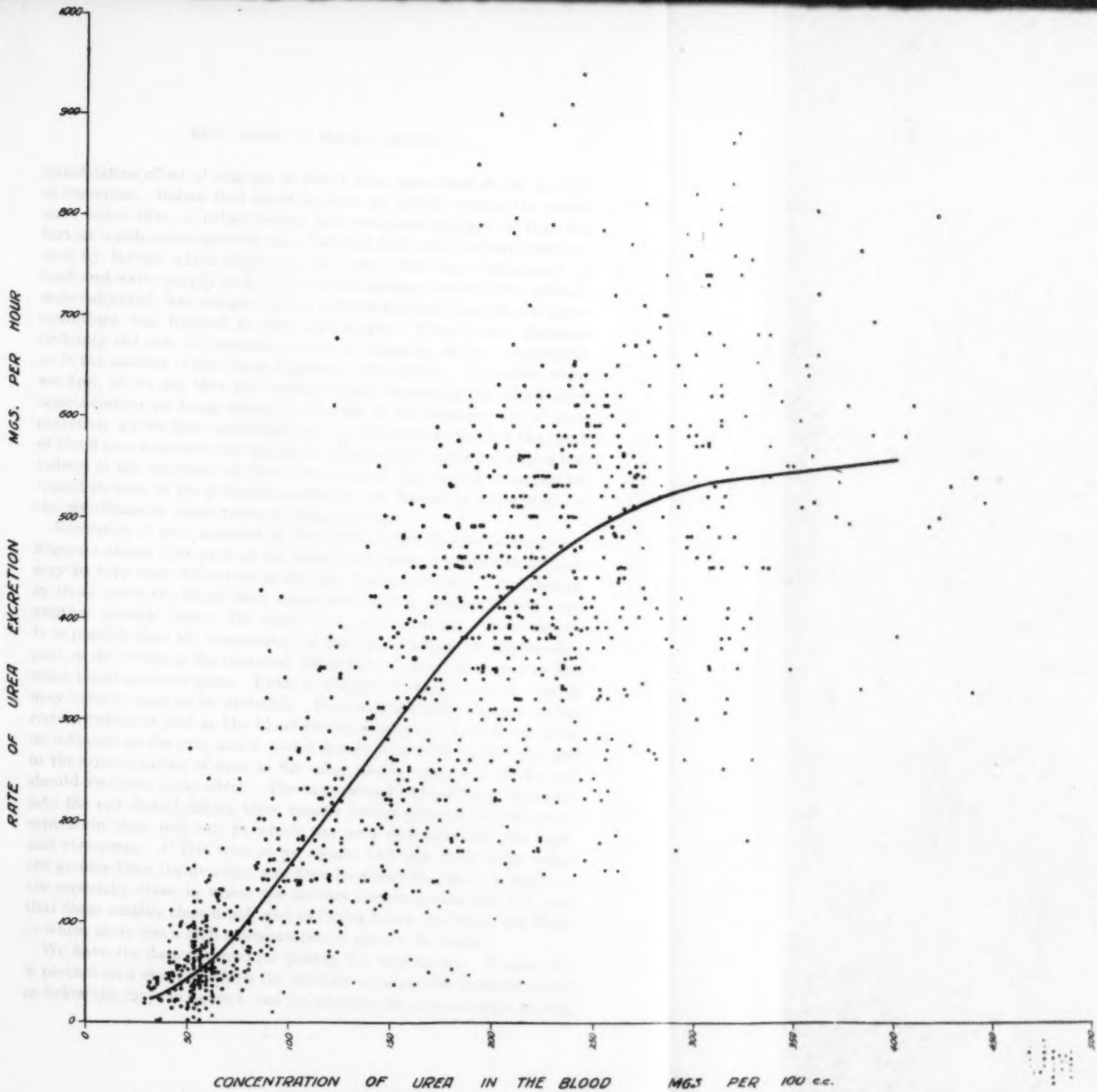

Fig. 1. The effect of changes in the concentration of urea in the blood on the rate of urea excretion in rabbits under standard conditions.

quantitative effect of changes in blood urea concentration on the rate of excretion. Before that could be done we should require the assurance either that all other factors had remained constant or that the factors which accelerated the rate had had their effect exactly neutralized by factors which depressed the rate. But mere uniformity in food and water supply and in the manipulations to which the animals were subjected, does not give us any right to suppose that either of these conditions was fulfilled in our experiments. These crude methods certainly did not, for instance, insure a constancy in the composition or in the amount of the blood supplied to the kidney. Therefore when we find, as we do, that by experimentally increasing the blood urea concentration we bring about an increase in the average rate of urea excretion, all we have accomplished is a demonstration that the level of blood urea concentration is a factor whose effect on the activity of the kidney in the excretion of urea is so marked that its influence can be traced in spite of the probable confusion and distortion introduced by the simultaneous inconstancy of other factors.

Regulation of urea excretion by the concentration of urea in the urine. Figure 1 shows that even at the same blood urea concentration there may be very wide differences in the rate of urea excretion. But though in these cases the blood urea concentration was constant, there was another possible factor, the urine urea concentration, which varied. It is possible that the inconstancy of this factor played an appreciable part in determining the observed differences in rates measured at the same blood concentration. From a strictly physical point of view, it may even be said to be probable. For we know that changes in the concentration of urea in the blood on one side of the kidney cell have an influence on the rate, and it would therefore seem likely that changes in the concentration of urea in the urine on the other side of the cell should also have some effect. The urea already taken from the blood into the cell should diffuse more rapidly into a urine of low urea concentration than into one in which the urea concentration was high, and vice-versa. If this were so we should find that those rates which are greater than the average, and thus lie above the curve in figure 1, are especially those in which the urinary concentration was low, and that those smaller than usual, and so falling below the curve, are those in which there was a high concentration of urea in the urine.

We have the data required for testing this hypothesis. If each rate is plotted on a chart in which the ordinate measures its distance above or below the curve in figure 1, and the abscissa the concentration of urea

in the urine, we shall find, if it is correct, that the points will group themselves on either side of a line running downwards from the left to the right of the chart.

This has been done in figure 2. There is no evident grouping. The points are scattered more or less uniformly all over the chart so that the curve of the average deviation coincides roughly with the zero line of the ordinate scale. Therefore the particular hypothesis we put forward is incorrect, and further, no other hypothesis of the manner in which the urea concentration of the urine influences the rate of urea excretion is valid, since the chart indicates that there is no appreciable effect at all. The same conclusion necessarily follows in regard to the possibility of changes in the volume of urine being a factor whose influence is of sufficient importance to be capable of demonstration under the conditions of our experiments.

DISCUSSION

In a recent monograph Cushny says,

> The formation of the glomerular filtrate is due to a blind physical force, the absorption in the tubules is equally independent of any discrimination, for the fluid absorbed is always the same, whatever the needs of the organisms at the moment (4).

Those who hold such views will expect to find some relation between the urea concentration or volume of the urine and the rate of urea excretion. On the other hand the demonstration that the rate is not appreciably affected by these factors will not be surprising to those who are inclined to believe that the mechanical factors influencing the action of the kidney may be coördinated and overruled by a power which adapts them at every moment to the needs of the organism as a whole. Our experience seems to favor this latter view for it shows that in the excretion of urea the kidney is in some way freed from subjection to the physical forces originating in the composition of the urine within its tubules. That urine is no longer a part of the organism but is already outside it, and the adaptability of the kidney to the internal requirements of the body would be prejudiced if it were forced to conform its action to variations in its external and inert environment.

But if the work of the kidney is constantly adapted to meet the requirements of the organism as a whole, how are the wide differences in rates of urea excretion measured at the same blood urea concentration to be explained? So far as its urea excreting function is concerned one

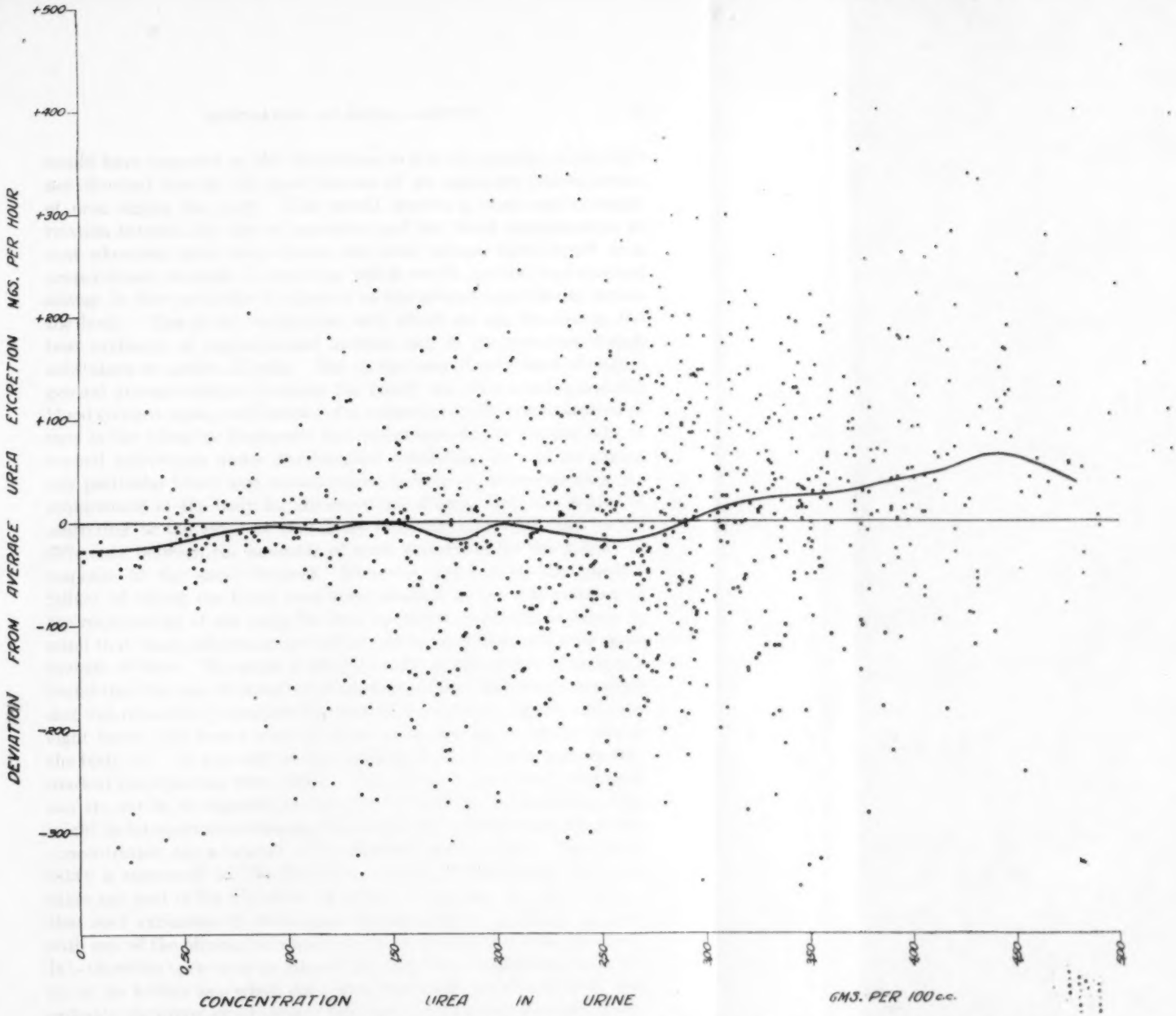

Fig. 2. The absence of any effect of changes in the concentration of urea in the urine on the rate of urea excretion in rabbits under standard conditions.

The ordinate measures + or − deviations from the average rate at the observed concentration of urea in the blood. The abscissa measures the concentration of urea in the urine.

The curved line is the average deviation at different levels of urea concentration in the urine. The fact that at all levels the average deviation approximates zero indicates that the concentration of urea in the urine has no appreciable effect on the rate of urea excretion.

would have expected on this hypothesis to find the activity of the kidney directed toward the maintenance of an optimum concentration of urea within the body. This would involve a close and constant relation between the rate of excretion and the blood concentration so that whenever more urea entered the blood stream there would be a proportionate increase in excretion which would prevent any marked change in this particular component of the general equilibrium within the body. This is the mechanism with which we are familiar in the heat exchange of warm-blooded animals and in the excretion of such substances as carbon dioxide. But in the case of urea we find only a general average relation between the hourly rate of excretion and the blood concentration, and instead of a constancy in the concentration of urea in the blood we frequently find variations of over 100 per cent in normal individuals under physiological conditions (5). If we regard any particular blood urea concentration in figure 1, as representing the requirement of the body for the excretion of urea, then the degree of scattering of the rates at that level indicates how wide may be the difference between the amounts of work performed by the kidney in response to the same demand. However, apart from the possible fallacy of taking the blood urea concentration as the sole measure of the requirement of the body for urea excretion, it should be borne in mind that these differences are only found in rates observed over short periods of time. We made a special study of this point in man and found that the rate of excretion of pre-formed urea added to a constant diet was remarkably constant for times of twenty-four, twelve and even eight hours, and bore a close relation to the amount of excess urea in the body (6). It was only in rates measured for one-hour periods that marked discrepancies were found. Now if these short-lived irregularities are not be to regarded as instances of a failure in regulation, they might be interpreted as showing that temporary variations in blood urea concentration are a matter of indifference to the body. This possibility is supported by the fact that we have no knowledge that urea takes any part in the metabolic functions of the body, nor any evidence that such variations in blood urea concentration as are found interfere with any of the physical or chemical states essential for vital processes. It is therefore open to us to suppose that the forces regulating the activity of the kidney as a whole may, over short time periods at least, subordinate the excretion of urea to the more pressing requirements of the moment arising from the need for the retention or excretion of substances of greater physiological importance than urea. It will be noted

that this conception implies that the functions of the kidney in the excretion of different substances are not entirely independent, so that a state of hyperactivity in the elimination of chloride, for instance, may be necessarily associated in some degree with increased work in the excretion of urea, in spite of the fact that no change may have occurred in the urea content of the tissues and blood. We have some experimental evidence, as yet incomplete, which seems to be in favor of this view, but this is a question which will require for its solution more direct evidence than any which we have as yet obtained. The immediately succeeding papers deal with the effect of factors of another variety and suggest another explanation for variations in rates measured at the same blood urea concentration.

CONCLUSIONS

1. In the rabbit the concentration of urea in the blood is an important factor in determining the rate of excretion of urea measured over short periods of time. Its effect, however, only becomes clearly apparent when the averages of many rates observed at different levels of blood urea concentration are compared, and at every level of blood concentration individual rates are found which are much higher or lower than the average.

2. Neither the concentration of urea in the urine nor the volume of urine are factors which appreciably influence the rate of excretion of urea under the conditions of our experiments.

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THE REGULATION OF RENAL ACTIVITY

II. Regulation of Urea Excretion by Anatomical Factors

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Although our immediate aim is the determination of the nature of the variable factors influencing urea excretion and although we accordingly adopted measures intended to exclude the influence of factors associated with the permanent structure of the kidney, it is necessary to consider to what extent we have failed in this endeavor and to attempt to define the degree to which anatomical peculiarities in the kidneys of the rabbits we used may have influenced our results. In this paper we have also considered some effects of anatomical factors on the rate of urea excretion in general.

It is conceivable that the rate of urea excretion is determined by only two factors, the blood urea concentration and the amount and quality of the urea secreting tissue of the kidneys. In that case differences in rates observed in a group of individuals in spite of constant blood concentration would arise from divergences in the structure of their kidneys. In that case also in the same individual the rate would be determined solely by the blood concentration. At the same concentration the rate would always be the same and at different concentrations we should find a constant relation between the rate of excretion and the level of blood urea concentration. It is, of course, not difficult to show that this conception is too narrow and rigid to account, even approximately, for the complexities of the process of urea excretion. In the same individual, for instance, rates may vary widely at the same blood concentration. We reproduce data from single rabbits in illustration of this point.

In these individual cases we see that the same kidneys manifest differences in rates measured at the same blood concentration; differences which are not appreciably smaller than those shown between the rates from the group of many different kidneys which are charted in

figure 1 of the preceding paper. It is therefore evident that such anatomical divergences as may have existed between the kidneys of this group had only a relatively insignificant influence in the production of these differences. The small and constant differences which minor structural variations induce are obscured by the large and variable differences arising from the operation of unknown factors.

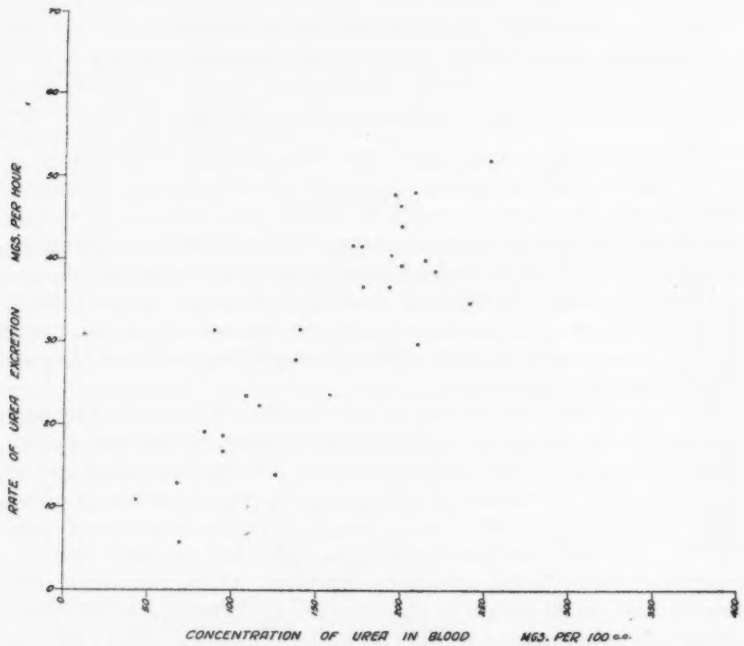

Fig. 1. Observations on a single rabbit showing that in the same individual the rate of urea excretion may vary widely though measured at the same or approximately the same blood urea concentration.

We have not made any detailed and systematic study of the degree of anatomical divergence which is required to produce a difference in function so marked that it will not be obscured by variable factors. From the practical point of view that is a question of great importance, but it would be best to undertake it when more is known as to the nature of these unknown factors and after means are devised under

which their action is controlled or rendered less variable. From the few observations we have made under the conditions of our present experiments, it would appear that within the same species it is only when differences between amounts of kidney tissue transcend the

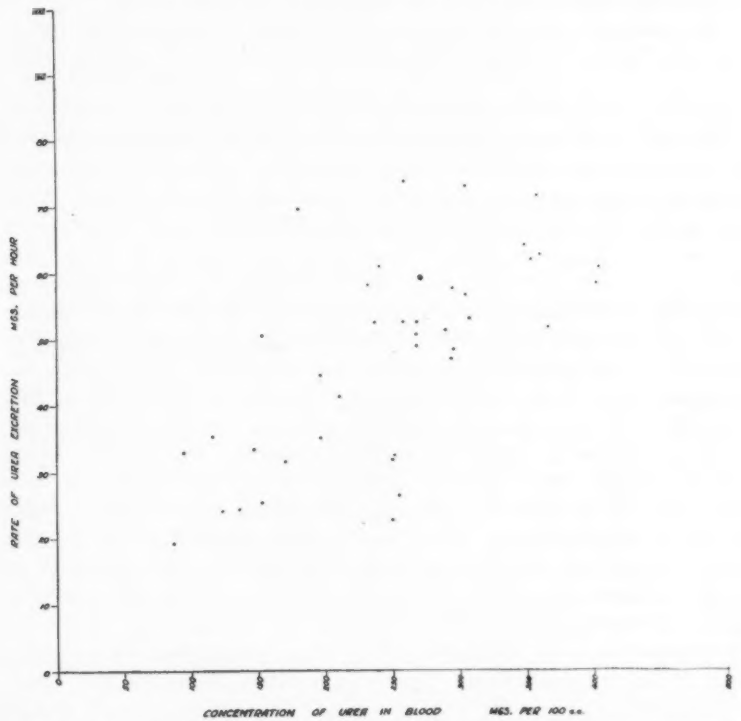

Fig. 2. Observations on a single rabbit showing that in the same individual rates may vary widely though measured at the same or approximately the same blood urea concentration.

range of normal adult variability, that a corresponding difference in function becomes clearly apparent.

In two unusually large rabbits, each weighing about 3000 grams, the average of twelve hourly rates of urea excretion was 421 mgms., the blood concentration averaging 197 mgms. per 100 cc. In four very small rabbits ranging from 350 to 515 grams, the average of

sixteen determinations carried out under the same conditions was only 130 mgms., although the urea concentration was 243 mgms. per 100 cc. Therefore marked differences in body weight, and so presumably in kidney weight, between animals of the same species, are sufficient to lead to quite pronounced differences in rates of urea excretion.

We used only medium sized animals in all other experiments, but it was not feasible to adopt a very rigid standard and it is probable that some part of the variability in rates measured at high blood concentrations arose from differences in the quantity of renal tissue in our animals.

We also could not be sure that the qualitative anatomical changes produced in the kidney by either general or local disease were not important factors in certain cases. Our rabbits unfortunately were not always in good health. A considerable number died from natural causes. In these, coccidiosis was usually the only gross lesion discovered. There was also a condition, frequently only temporary, characterized by loss of weight and by a rise in blood urea concentration, usually most pronounced in those animals in which the greatest emaciation had occurred. But there was not by any means always an appreciable defect in the urea excreting capacity of the kidney in this condition. Indeed, we sometimes saw kidney function continue undisturbed almost up to the very moment of death. On the other hand, we occasionally came across animals in which renal activity was at a level much lower than the average, although they presented no signs or symptoms of disease. Even post mortem examination of the kidneys did not give any sure guidance in determining the normality or abnormality of our animals. This was because we used the rabbits in repeated experiments and only examined the kidneys when they died from natural causes. The acute diffuse degenerative renal lesions which were found in some of these cases could rarely be associated with functional changes, since the time at which they originated could not be fixed. There was also, of course, the usual high percentage of cases of so-called spontaneous nephritis, a focal interstitial lesion which did not appear to have any appreciable effect on function. Chemical and microscopical examination of the urine would perhaps have been the best way to exclude animals with diseased kidneys, but as is well known, albuminuria is almost constant in caged rabbits, and from the nature of the urine casts are often difficult to find.

In the end we decided that, since we had no very reliable criterion, it would be better to make no attempt at selection at all. The only observations we have rejected are those made on animals which died

during the course of the experiment or very shortly afterwards. In preliminary work of this sort in which a considerable mass of data has been collected, the inclusion of all observations is perhaps the most satisfactory method. For though we thereby lose precision and detail in our deductions, we may at least feel sure that any general conclusions which may be reached are unbiassed by a selection, necessarily more or less arbitrary, from which it would have been hard to exclude the personal factor.

We have confined discussion so far to the influence of anatomical factors as possible causes of differences between rates of urea excretion observed in animals of approximately the same size and measured at the same blood urea concentration, that is to say as possible explanations of the high degree of scattering shown in figure 1 of the first paper. It

TABLE 1

Average rates of urea excretion in the rabbit and in man at different levels of blood urea concentration

UREA IN 100 CC. OF BLOOD	UREA IN ONE HOUR'S URINE	
	Rabbit	Man
mgms.	*mgms.*	*mgms.*
40	32	1100
50	45	1550
60	59	2100
70	79	2600
80	102	3150
90	125	3685

is only from this aspect that any influence they may have is of moment so far as our present investigation is concerned. We have shown that from this point of view their effect is small and uncertain.

But from a wider standpoint the anatomical factor of kidney size is of primary importance in regulating the rate of urea excretion. Other factors may regulate the activity of the kidney so that a quite wide range of rates is found at any given blood urea concentration, but the value of the average rate round which these variations occur is determined by the size of the kidney. This becomes apparent on comparing the average rates yielded by kidneys of widely different sizes, as for instance the kidney of man and of the rabbit. In the above table the contrast is carried only as far as a blood concentration of 90 mgms.,

since no average based on a sufficient number of observations was obtained from man beyond that point.

It is here shown that the average rate in man is rather more than thirty times greater than in the rabbit. Approximately a man weighs thirty times more than a medium sized rabbit. From the relation which exists between body weight and kidney weight there is presumably about thirty times more renal tissue in man than in the rabbit. The order of magnitude of the average rate of urea excretion appears therefore to be a function of the size of the kidney.

The form of the curve of the rate of urea excretion is different from the similar curve in man. The form of the curve of the ratio between the urea content of the urine and of the blood is also necessarily different. This ratio is an expression we have found convenient, especially for statistical purposes. It is obtained by dividing the urea content of one hour's urine by the urea content of 100 cc. of blood. When the rates charted in figure 1 of the first paper are plotted as ratios, the curve of the average ratio is seen to rise to a maximum and then to fall (see fig. 3). The similar curve in man shows no such declension.

But in man the curves were only carried to a concentration of 100 mgms. per 100 cc. while in the rabbit they are carried as far as 400 mgms. per 100 cc. If we had forced the blood urea concentration as high in man as in the rabbit the analogous curves might have followed the same course. Within the concentrations at which they can be compared, there is no essential difference.

The gradual rise of the ratio curve up to a certain level of blood concentration and its subsequent decline at still higher levels resembles the type of curve obtained when the work output of a muscle is charted under successively increasing loads. The rise in the ratio might then be regarded as the effect of strain on the activity of the kidney and its fall as the effect of over-strain.

But the mode of energy transmission is so radically different in muscle as compared with kidney tissue, that this analogy may be misleading. It is also necessary to be cautious in drawing deductions from the form of curves constructed from such heterogeneous data as ours. It seemed possible, for instance, that those observations made when the blood urea concentration was exceptionally high might have been drawn in the main from animals whose kidneys were diseased. The fall in the curve would be much more convincing if it were shown to occur in animals whose reaction to lower urea concentrations had been shown to be normal.

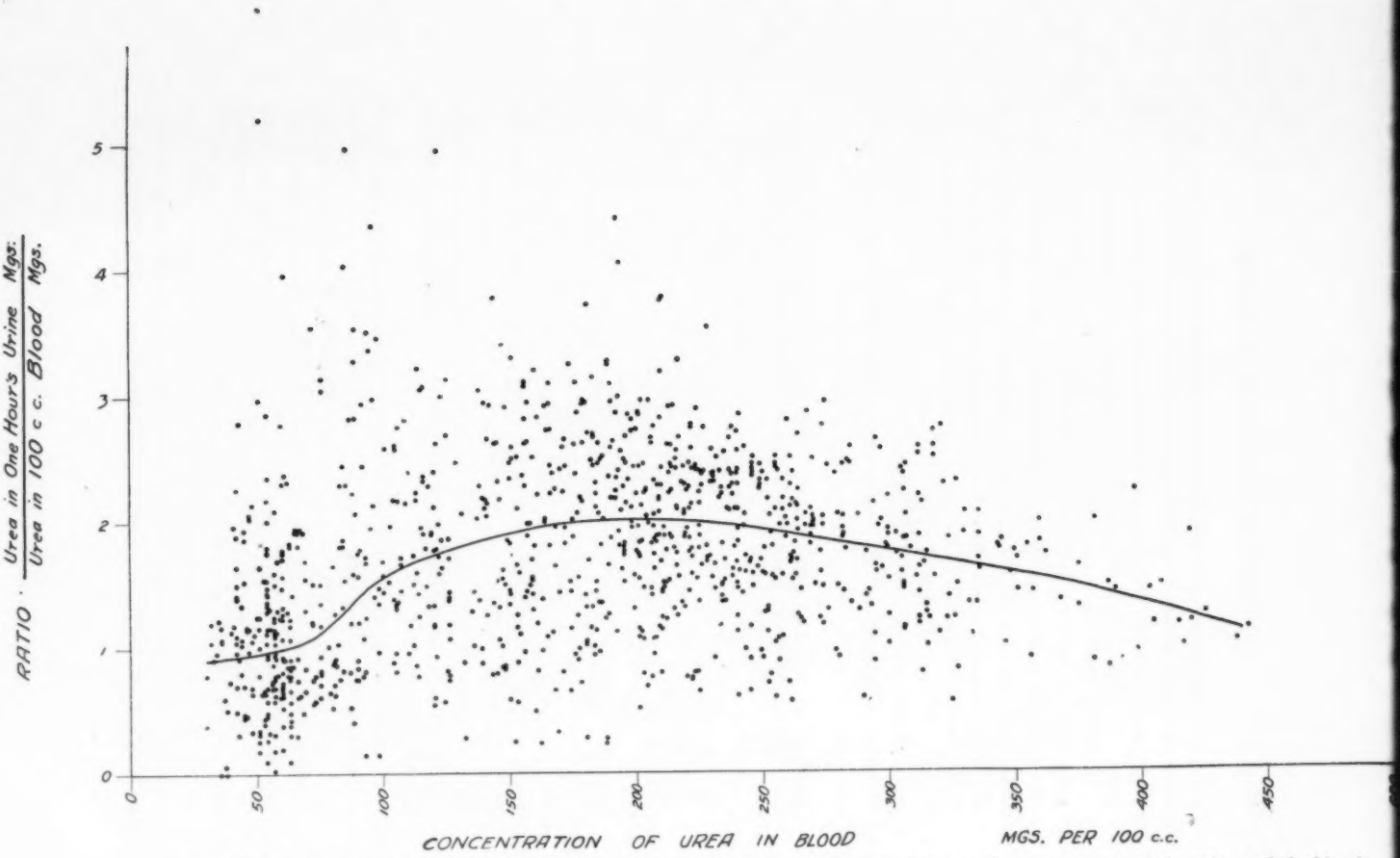

Fig. 3. The effect of changes in the concentration of urea in the blood on the ratio between the urea content of the urine and the blood

Unfortunately we have few animals on which observations were made at several widely differing blood concentrations, but there is one group of seven rabbits on which experiments after no urea, 5 grams and 10 grams of urea were carried out. The averages are given in table 2.

These figures lend no support to the supposition that the fall of the ratio curve in figure 3 at blood concentrations over 225 mgms. can be due entirely to the inclusion of animals with diseased kidneys. For this group, whose kidneys reacted normally at low and medium levels, shows a quite definite and constant decrease in the ratio at concentrations over 300 mgms. as compared with those measured at about 200 mgms. We may therefore conclude that the decline of the curve of the ratio in the group chart represents, qualitatively at least, the true reaction of the normal kidney to very pronounced and sudden increases

TABLE 2

A comparison of average rates of urea excretion and of average ratios from the same group of animals as measured at low, medium and high blood urea concentrations

AMOUNT OF UREA ADMINISTERED	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in one hour's urine	Urea in 100 cc. blood	Ratio	Urea in one hour's urine	Urea in 100 cc. blood	Ratio	Urea in one hour's urine	Urea in 100 cc. blood	Ratio	Urea in one hour's urine	Urea in 100 cc. blood	Ratio
grams	*mgms.*	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*	
0	48	64	0.62	81	71	1.10	79	70	1.05	104	73	1.53
5	134	145	0.99	333	190	1.84	431	207	2.18	409	219	1.93
10	261	210	0.96	510	307	1.68	671	375	1.69	668	382	1.83

in blood urea concentration, though quantitatively it may be somewhat exaggerated by occasional instances of decreased function due to pathological causes.

Though the hypothesis of over-strain is thus supported there is no reason to suppose that this decrease in the ratio between urea excreted and urea still to be excreted is the result of a process similar to the "fatigue" of muscle. There is no evidence that the products of the metabolic activities of the kidney accumulate so as to hamper the work of the secreting cells. And there is no relation between the time during which a kidney has been under strain and the degree of decrease in the ratio. In the above example, for instance, the decrease after 10 as compared with 5 grams of urea is more marked in the second and third periods than in the last. And in experiments on rabbits in which the

blood urea concentration was kept for days and even weeks at an almost continuously high level by repeated doses of urea, we never found any change in function which could be interpreted as the result of fatigue.

There is, however, a factor which would account for a decrease in ratios at very high blood urea concentrations. When the blood concentration is forced higher and higher by the administration of increasing quantities of urea, theoretically at least, a concentration will at last be produced at which the rate of urea excretion will have reached the maximum to which the quantity of renal tissue present is capable of attaining. There the curve of the rate must cease to rise and must thereafter move horizontally. There, also, the curve of the ratio must commence to decline in proportion to every further increase in blood urea concentration. That concentration represents the amount of work in urea excretion which taxes all the urea secreting tissue of the kidney to its utmost capacity.

We believe that the flattening of the rate curve and the fall in the ratio curve at concentrations above 225 mgms. per 100 cc. do, in fact, represent the effect of the limiting factor of kidney size. That in our curves the change in direction should not be sharply defined is to be expected, when it is remembered that they are averages compiled from data on a considerable number of animals, each with its own individual renal capacity.

The only reason for doubt lies in the possibility that the diminution in the relative activity of the kidney represented by the change in the direction of the curves might arise from secondary effects and not as a direct result of an overburdening of the kidney tissue by the urea itself. For though urea is perhaps of all substances the one to which the body is most indifferent, yet when such enormous quantities are given as are required to raise the blood urea concentration to over 225 mgms. per 100 cc. in subjects whose kidneys are normal, we are doing more than increase the work the kidney is called on to perform in the excretion of urea. These great concentrations more or less suddenly induced, must tend to disturb the balance of molecular concentration in the tissues. It has been shown also that the intravenous injection of 80 per cent urea solutions leads, in rabbits, to an alteration in the haemoglobin percentage of the blood, presumably due to an increase in its water content (1). And in man symptoms such as headache and inability for physical or mental exertion are induced by the administration by mouth of 100 to 120 grams of urea, amounts which, though they in no case raised the blood urea concentration much over 200 mgms. per 100

cc., were yet followed by a reduction in the haemoglobin percentage (2). Further, rabbits are susceptible to poisoning by ammonia derived from the bacterial decomposition of unabsorbed urea reaching the large intestine (3). A number of our animals died from this cause after 5 grams of urea (4) and apparently the greater the quantity of urea given the more frequently does ammonia poisoning occur. In the above group of seven, only two escaped after doses of 15 grams. Instances of non-fatal ammonia poisoning were occasionally seen, so that it is not unlikely there were a number of minor undetected cases.

All these secondary effects of large doses of urea may have combined to embarrass the work of the kidney, and if we had not seen so many instances of urea excretion remaining undisturbed under procedures involving much more serious general disturbances than the hypothetical ones we have mentioned, we should have been inclined to attach more importance than we do to this possible explanation.

Though the change in direction of the rate and ratio curves is due to the limitation on function imposed by the structural capacity of the kidneys yet under physiological conditions the reaction of the kidney to its environment is in no way influenced by this ultimate anatomical limitation. This is only a potential factor in the regulation of renal activity, operative in extreme cases of reduction of kidney size by disease, or in the entirely artificial condition arising from the ingestion of large quantities of urea. The normal kidney has a possible range of action much greater than that which it actually covers. For if the average blood urea concentration of the rabbit be taken as 30 mgms. per 100 cc., the fact that the ratio continues to increase up to a level of 225 mgms. indicates that the capacity of the kidney is about seven times greater than that which is just sufficient. In anatomical terms, it shows that there is seven times more renal tissue than is ordinarily called into full action.

CONCLUSIONS

1. The size of the kidney determines the order of magnitude of the average rate of urea excretion at all blood urea concentrations.

2. In medium sized rabbits such differences as presumably exist between the amounts of renal tissue they possess are too small to account for any but a small part of the marked differences which occur between rates of urea excretion measured at the same blood urea concentration.

3. There is no marked increase in the average rate of urea excretion in rabbits when the blood urea concentration rises higher than 225 mgms. per 100 cc. This is interpreted as indicating that about that level of blood urea concentration, the activity of the kidney becomes limited by the factor of kidney size.

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THE REGULATION OF RENAL ACTIVITY

III. Regulation of Urea Excretion by Unknown Factors

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The excretion of urea is regulated not only by the blood urea concentration and by the amount of active secreting tissue in the kidney, but also by other and unknown factors. The operation of these factors is demonstrated by the occurrence in the same animal of differences between rates measured at the same blood concentration, and their scope of action is indicated by the extent of these differences. In this paper an analysis of some general features of these differences has been made in the hope that some information might be obtained in regard to the mode of action of the factors through whose intervention they arise, and especially in order to obtain a foundation for deductions as to the method best adapted for a more particular investigation of their nature.

The effect of the unknown factors varies progressively in consecutive observations. It will be remembered that in each experiment four consecutive collections of urine and of blood were made. When no urea was given, the blood urea concentration remained at about the same level throughout each of these four periods, and one might have confidently expected that the rate of urea excretion, though exhibiting irregular fluctuations, should on an average also have remained at approximately the same level since no food had been taken for seventeen hours and there was no apparent reason why the formation and elimination of urea should not have proceeded at a more or less even pace.

We were therefore surprised to find that the rate of urea excretion showed a pronounced and progressive increase in each successive period of the experiment. In table 1 we give the averages in each period of the rate of urea excretion and blood urea concentration and of the ratio between the urea content of the urine and of the blood. These were obtained from forty-three experiments on a group of thirty-four rabbits which received neither water nor urea. These averages are given in

table 1 charted in figure 1 and the details for each animal are given in table 2 at the end of the paper.

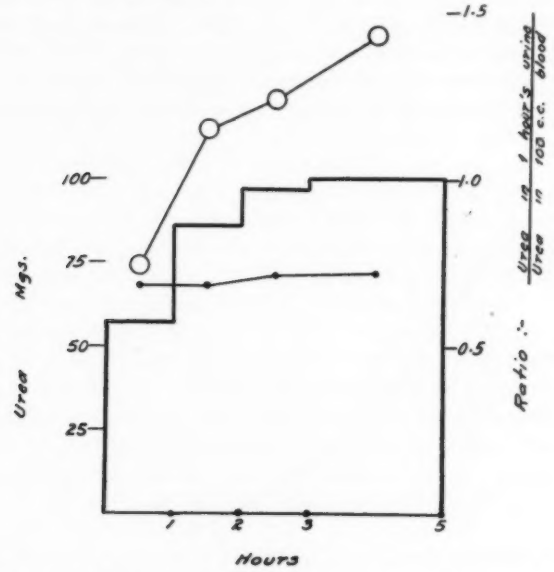

Fig. 1. Showing the increase in the rate of urea excretion in each successive observation, in spite of an approximate constancy in blood urea concentration.

The hourly rate of urea excretions represented by the blocked areas. The blood urea concentration is shown by dots joined by lines. The values for both the rate and the blood concentration are given by the scale on the ordinate at the left of the chart.

The ratio between the urea in one hour's urine and the urea in 100 cc. of blood is given as circles joined by lines. The value is shown by the ordinate at the right of the chart.

TABLE 1

Rate of urea excretion in consecutive observations during which the blood urea concentration remained constant. Averages of 43 experiments on a group of 34 rabbits

PERIOD	UREA IN ONE HOUR'S URINE	UREA IN 100 CC. BLOOD	*RATIO:* $\frac{\text{UREA IN ONE HOUR'S URINE}}{\text{UREA IN 100 CC. BLOOD}}$
	mgms.	*mgms.*	
First hour	57	68	0.74
Second hour	86	68	1.15
Third hour	97	71	1.24
Fourth and fifth hours	100	72	1.43

The blood urea concentration remains practically constant, varying only between 68 and 72 mgms. and yet the hourly rate of excretion increases from 57 mgms. in the first period to 86 mgms. in the second, to 97 in the third and to 100 mgms. in the last. This means that in some way an increase in the urea excreting activity of the kidney had gradually taken place for at its highest point, where 100 mgms. are excreted per hour or 2.4 grams per 24 hours, we have a rate which is considerably

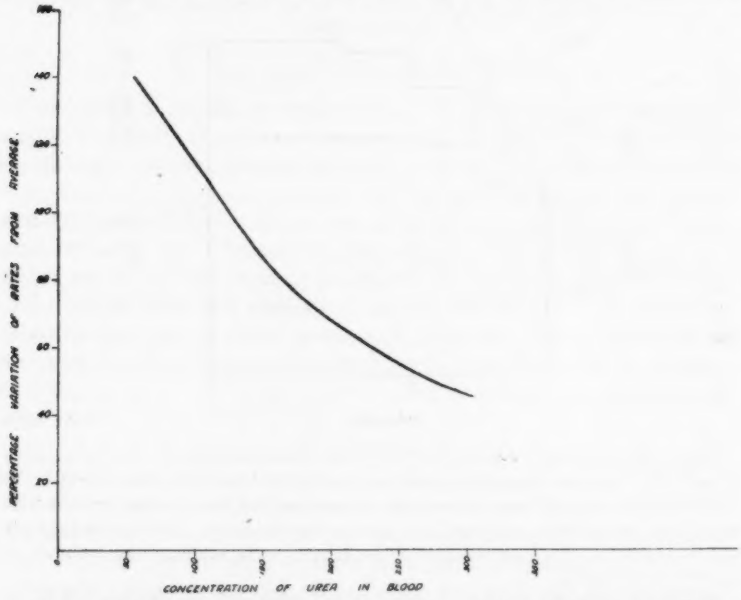

Fig. 2. Showing the decrease in the variability of the rate of urea excretion as the concentration of urea in the blood increases. Variability is measured by the percentage standard deviation from the average rate of urea excretion.

greater than that shown by medium sized rabbits for the first 24 hours of abstinence from food and water, when they are left undisturbed and are not subjected to manipulation.

This progressive rise can be attributed neither to anatomical factors nor to changes in blood urea concentration. It is an example of the effect of unknown factors which so regulate the action of the kidney as to lead to an increasing efficiency in the excretion of urea with the passage of time from the commencement of the experiment.

Conditions which influence the variability in urea excretion produced by unknown factors. A knowledge of the conditions under which the operation of these unknown factors is most clearly manifested is of importance in the selection of the experimental conditions best adapted for their study; for an experimental intensification of a factor suspected of belonging to this group may perhaps only yield a significant deviation from the usual mode of action of the kidney if the control standard has been established under conditions in which the kidney is most sensitive to the influence of the factor in question.

Undoubtedly the most important of these conditions is that the time over which kidney function is measured should be short. The unknown factors produce only evanescent fluctuations in kidney activity which rise and fall and quickly tend to counterbalance one another (1). Their action must therefore necessarily be investigated over short time intervals such as we have adopted.

The level of blood urea concentration is another condition which has a considerable influence. The absolute variability at each level of blood concentration is shown in the degree of deviation of the points above and below the curves of the average in the rate and ratio charts. But the true measure of variability is, of course, relative and not absolute. In figure 2 the curve of the percentage standard deviation from the average rate is given.

It will be noted that the variability is greatest when the blood urea concentration is low, and decreases the higher it rises.

The time relationships of consecutive observations also influence the variability, for during the first hour the variability is higher than in subsequent periods. Thus in thirty-five experiments on a group of twenty-seven rabbits, whose blood urea concentration remained at a constant level, the standard deviation of the ratio was 43 per cent of the arithmetical mean in the first period, and fell to 34 per cent, 35 per cent and 36 per cent respectively for the three remaining periods.

DISCUSSION

This analysis of the data given in the first paper of this series allows us to form some conclusion as to the methods most likely to prove effective in an inquiry into the nature of the factors we have grouped under the term unknown.

In the first place, the great variability of rates or of ratios measured at constant blood concentration under our standard conditions makes it plain that statistical methods must be applied before weight can be

attached to deviations found under experimental conditions. It will manifestly not be enough to compare single experiments. The averages of groups must be the unit of comparison and the degree to which chance might account for any observed difference between averages must be calculated and taken into account.

We have shown that it is highly probable that in certain cases anatomical factors played a part in the production of such differences between rates or ratios in different animals as were not due to variation in blood urea concentration. Since we wish to eliminate all factors associated with structural peculiarities, we must obtain our average results on a group of animals under standard conditions and then repeat the work on the same group under, as far as possible, the same conditions except for the introduction of the factor whose effect we wish to test.

The remarkable increase in rates and ratios over consecutive hours of bleeding and catheterization indicates that in watching for the effect of any experimental factor, we must not be satisfied with the average rate over the whole five hours of observation, but must also attach importance to any statistically significant deviation from the progressive increase in successive hourly rates and ratios, which we have found to be characteristic of the mode of regulation under the conditions adopted as our standard.

Finally, we may expect to find the kidney more susceptible to the effect of the factors under investigation over short time periods when the blood urea concentration is low and particularly during the first period of observation.

CONCLUSIONS

1. There is a progressive increase in the rate of urea excretion in consecutive observations on rabbits subjected to catheterization and bleeding, so that at the last the rate is nearly twice as great as at the commencement of the experiment and exceeds the rate yielded by the rabbit under the same conditions except for the absence of handling. This increase in the activity of the kidney occurs in spite of the absence of any change in blood urea concentration.

2. The variability of rates or ratios measured at the same blood urea concentration is greater the shorter the time of observation, decreases as the blood urea concentration increases, and in a series of consecutive observations is most pronounced during the first.

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TABLE 2

Control experiments. No urea

RABBIT NO.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood
	mgms.	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*	
59*	27	50	0.53	38	52	0.75	50	55	0.90	60	59	1.03
65*	112	126	0.91	167	125	1.33	174	127	1.39	197	137	1.45
66*	44	58	0.76	64	56	1.16	59	56	1.06	81	54	1.47
67*	62	55	1.14	85	57	1.50	97	56	1.75	98	57	1.75
68*	34	40	0.80	40	38	1.10	29	44	0.70	66	50	1.31
69*	56	59	1.01	86	61	1.30	102	63	1.51	107	63	1.65
70*	18	43	0.41	55	45	1.22	87	45	1.47	95	45	2.14
71	27	54	0.49	44	57	0.76	52	57	0.92	76	60	1.26
72*	43	65	0.65	61	66	0.88	67	65	1.03	78	66	1.65
73	37	54	0.69	64	57	1.12	83	54	1.53	127	54	2.35
80	67	90	0.75	125	65	1.94	144	62	2.32	167	60	2.77
82	215	150	1.43	351	50	2.34	321	164	2.02	lost	lost	lost
83	177	126	1.40	199	136	1.45	229	147	1.55	221	148	1.50
85	143	120	1.34	209	117	1.79	252	120	2.11	216	121	1.78
86	150	156	0.97	177	153	1.16	177	141	1.26	203	150	1.35
87	155	146	1.07	191	138	1.39	302	141	2.14	315	138	2.28
88	18	55	0.33	41	56	0.74	41	56	0.74	71	57	1.24
89	89	60	1.47	119	66	1.80	126	66	1.91	154	54	2.85
90	32	45	0.70	45	47	0.97	61	50	1.24	72	54	1.33
91	38	56	0.68	79	54	1.47	63	57	1.10	91	57	1.59
92	37	48	0.64	73	54	1.36	62	54	0.77	94	60	1.56
93*	27	39	0.66	39	38	1.05	39	45	1.15	59	55	1.25
94	23	53	0.44	21	53	0.40	40	60	0.68	13	60	0.20
95	81	90	0.90	154	93	1.67	168	112	1.50	62	160	0.50
96	35	44	0.80	80	41	1.96	83	45	1.85	62	54	1.15
97	29	42	0.70	45	44	1.02	68	45	1.52	87	45	1.93
98	0	38	0.00	47	44	1.06	52	46	1.14	98	48	2.13
99	54	49	1.10	54	47	1.15	60	54	1.11	63	51	1.24
100	0	36	0.00	2	38	0.06	9	51	0.18	16	48	0.34
101	11	57	0.19	17	51	0.32	18	57	0.32	5	54	0.10
102	7	63	0.10	2	57	0.03	25	54	0.46	31	60	0.53
103	43	42	1.04	69	42	1.64	58	44	1.33	83	51	1.64
104	33	48	0.69	40	51	0.79	46	58	0.79	54	60	0.91
105	13	51	0.26	36	57	0.63	46	69	0.66	69	75	0.93
Averages....	57	68	0.74	86	68	1.15	97	71	1.24	100	72	1.43

* The asterisk indicates that in the case of the animals whose number is so marked the results given are the average of two experiments.

THE SALIVARY FACTOR IN RELATION TO DENTAL CARIES

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The power of the saliva to maintain a protective function over the buccal cavity and teeth has long been a subject for investigation, but little has been proven. That one of the functions of this fluid is to protect and benefit in some way the tissues which it continually bathes is sine qua non and the study of the saliva is an important phase in the understanding of the etiology of dental caries.

Miller (1) in 1890 suggested that in the saliva

> the great amount of carbonate of the alkalies is specially noteworthy; it imparts a strong alkaline reaction to the animal saliva, and is well calculated to neutralize such acids as may be found in the animal mouth, thus tending to prevent the appearance of caries.

Acids were considered the arch enemies of the teeth and it was a natural trend of research workers in dentistry to look for something to counteract any acidity in the mouth.

Marshall (2), however, in 1915 stated that the saliva is amphoteric and therefore for it to be a factor in protecting the teeth it must neutralize either acid or alkaline substances as taken into the mouth, and that it is the degree of this power to maintain a neutrality in the mouth which is indicative of susceptibility to caries.

He promulgated a theory of a "salivary factor" as an index of immunity from caries, which Gies and coworkers (3) could not substantiate by any of their work.

This report deals with a like investigation to find out what relation there may be between the salivary factor and the incidence to dental caries.

Marshall's procedure was followed using 10 cc. of the sample and titrating immediately after collection with N/200 NaOH and N/200 cc. HCl. Phenolphthalein and paranitrophenol were used as indicators. Paraffin was chewed as a stimulus for the activated saliva.

The saliva in all cases was diluted with freshly distilled water. This was done because in some instances the saliva was so dark colored that it would have been impossible to detect any color change, and it was always difficult to obtain more than 10 cc. of resting saliva from any patient.

All of the subjects were of about the same age, ten to fifteen years. These limits were set, as it is between these years that incidence to decay is most active, and 1500 children visiting the Infirmary every month for treatment made careful selection of cases comparatively easy. Those children with occasional fillings were discarded, as were also those with only one or two cavities. Absolute immunity and excessive decay only were chosen for the work to be done.

Note was taken of sex, general appearance, nationality, cleanliness of mouth, the time after a meal at which the saliva was collected and the character of the saliva. The resulting figures for each case were referred then to these things.

The patient was left alone during collection of the sample so that there would be no embarrassment nor any outside psychical excitation. As near normal conditions as possible were sought.

The following table is given as a fair illustration of the results ob-

TABLE 1

PATIENT				RESTING SALIVA			ACTIVATED SALIVA			SALIVARY FACTOR
No.	Sex	Age	Dental condition	Alkali	Acid	T. N.	Alkali	Acid	T. N.	
		years								
48306	M	11	Immune with care	3.5	6.1	9.6	0.3	19.7	20.0	48.00
48367	F	11	Immune	1.3	3.5	4.8	1.6	5.5	7.1	67.60
48234*	M	14	Immune	1.4	3.3	4.7	0.8	9.7	10.5	44.76
48291*	F	15	Immune with care	3.8	6.2	10.0	0.7	23.0	23.7	42.11
48148	F	12	Immune with care	1.0	4.5	5.5	0.4	13.2	13.6	40.44
48214	M	13	Immune	1.1	3.1	4.2	1.0	10.3	11.3	37.16
45928	F	13	Immune with care	3.0	2.1	5.1	0.6	4.1	4.7	108.50
45642	F	12	Immune with care	4.4	7.5	11.9	1.4	19.5	20.9	56.93
1962P*	M	14	Immune	5.8	15.7	21.5	2.7	35.2	37.9	56.72
1962S†	F	12	Immune with care	0.4	3.7	4.1	0.3	8.5	8.8	46.65
45861*	F	10	Immune with care	0.3	5.5	5.8	0.6	13.4	14.0	41.42
48569	F	14	Immune with care	1.7	2.4	4.1	1.1	7.6	8.7	47.12
Averages				2.88	5.3	7.35	0.9	14.1	15.0	52.43

* Activated saliva alkaline to phenolphthalein.

† Activated and resting saliva alkaline to phenolphthalein.

tained from immune mouths. The columns headed "alkali" mean the amount of alkali used to neutralize the sample or the acidity of the sample. "Acid" designates the amounts of acid used or the alkalinity of the sample.

No constancy of results can be detected in the preceding table. The figures given for acidities, alkalinities, total neutralizing powers and salivary factors vary widely.

For example, the acidity column of resting saliva gives outside limits of alkali used as 5.8 cc. and 0.3 cc. with the average 2.88 cc. Wider

TABLE 2

PATIENT				RESTING SALIVA			ACTIVATED SALIVA			SALIVARY FACTOR
No.	Sex	Age	Dental condition	Alkali	Acid	T. N.	Alkali	Acid	T. N.	
		years								
47084K*	F	14	Decay	5.5	3.7	9.2	2.5	3.1	5.6	164.26
47084P	F	11	Decay with care	0.7	1.8	2.5	0.5	7.7	8.2	30.48
48374	M	11	Decay	0.7	2.0	3.7	0.2	2.4	2.6	103.82
8431H	F	14	Decay	1.2	4.4	5.6	0.6	4.5	5.1	109.01
19564*	F	12	Decay with care	3.2	5.4	8.6	0.7	20.8	21.5	40.00
9732TP*	F	13	Decay	1.2	2.4	3.6	0.8	5.3	6.1	69.01
8362	M	12	Decay with care	1.1	2.8	3.9	0.6	7.2	7.8	50.00
1906A	M	12	Decay with care	0.4	2.7	3.1	0.5	4.5	5.0	62.00
46873†	M	13	Decay	2.2	3.7	5.9	0.1	6.4	6.5	90.76
1908A	M	12	Decay with care	1.1	5.2	5.3	0.1	10.7	10.8	49.07
1965A†	M	12	Decay	0.3	1.4	1.7	0.1	4.4	4.5	38.00
Averages				1.66	3.22	4.82	0.6	7.7	8.3	64.96

* Activated saliva alkaline to phenolphthalein.

† Activated and resting saliva alkaline to phenolphthalein.

variations than these are found in all but the acidity column of activated saliva.

Table 2 shows the results from carious mouths.

This table shows a similar disregard for constancy. An example of the inconstancy here is the salivary factor column, where the average factor is 64.96, and one of 164.26 and one of 38.00 are found with variations from these in between.

Comparison of tables 1 and 2 displays no striking difference. The averages of all columns of the immune table except that of salivary factors are higher than those of the caries table, but in every column

of the immune cases there will be found figures below the average of the corresponding column of carious cases and likewise in every column of the caries table there will be found figures above the average of the corresponding column of immune cases. Table 3 illustrates this.

TABLE 3

CASE NO.	DENTAL CONDITION	RESTING SALIVA			ACTIVATED SALIVA			SALIVARY FACTOR
		Alkali	Acid	T. N.	Alkali	Acid	T. N.	
47084	Decay	5.5	3.7	9.2	2.5	3.1	5.6	164.26
19564	Decay with care	3.2	5.4	8.6	0.7	20.8	21.5	40.00
48214	Immune	1.1	5.3	4.2	1.0	10.3	11.3	37.16
48928	Immune with care	3.0	3.22	5.1	0.6	4.1	4.7	108.50
1962S	Immune with care	0.4	3.1	4.1	0.3	7.5	8.8	46.66
45642	Immune with care	4.1	2.1	11.9	1.4	19.5	20.9	56.93
Average	Immune	2.88	3.7	7.35	0.9	14.1	15.0	52.43
Average	Decay	1.66	7.5	4.82	0.6	7.7	8.3	64.9

This table also illustrates the fact that while the average salivary factor for carious mouths is higher than that of immune mouths, both factors are below 80+ (Marshall's line of demarcation between an immune and carious mouth), and again that factors of carious cases may be below the average factor of immune cases and factors of immune cases may be above the average carious factor.

Some samples of saliva, both resting and activated, were alkaline to phenolphthalein, but this phenomenon occurred with such frequency in both immune and carious cases that no importance can be attached to it. Table 4 shows the indiscriminancy with which it occurred. The resting or activated or both kinds of saliva of all of these examples were alkaline to phenolphthalein.

TABLE 4

CASE NO.	AGE	DENTAL CONDITION	SALIVARY FACTOR
	years		
48234*	14	Immune	44.76
47084K*	14	Decay	164.26
1962S†	12	Immune with care	46.66
19564*	12	Decay with care	40.00
46873†	13	Decay	90.76

* Activated saliva alkaline to phenolphthalein.

† Activated and resting saliva alkaline to phenolphthalein.

The following table shows very well the lack of constancy of sex and age in regard to the salivary factor. Numbers 48306 and 48569, a boy and a girl with three years difference in age, vary hardly at all, and numbers 1906A and 1905A, both boys of the same age, give very different salivary factors.

TABLE 5

CASE NO.	SEX	AGE	DENTAL CONDITION	SALIVARY FACTOR
		years		
48306	M	11	Immune with care	48.00
48569	F	14	Immune with care	47.12
1906A	M	12	Decay	62.00
1905A	M	12	Decay	38.00

The general appearance of all the subjects was that of ordinary, normal boys and girls. Slovenly appearance was not displayed more frequently in one sort of a case than in the other. Carious mouths were found in both overdeveloped and underdeveloped as well as in normal children. Age, sex and condition of the mouth had no connection with the nervous embarrassment of the patient or the resulting salivary factor.

Table 6 gives examples of the inconstancy of salivary factors with regard to general appearance.

TABLE 6

NATIONALITY	CASE NO.	AGE	SEX	DENTAL CONDITION	SALIVARY FACTOR
		years			
Swede	19564	12	F	Decay with care	40.00
French	9732TP	13	F	Decay	59.00
Irish	8362	12	M	Decay with care	50.00
Irish	47084K	14	F	Decay	164.26
Irish	48148	12	F	Immune with care	40.41
Jewish	48928	13	F	Immune	108.50

The first two cases cited were unusually attractive, healthy, wholesome looking girls, but they had atrociously bad teeth. Each girl had six sound teeth in her head. Number 8362, who also had very poor teeth was a sickly undernourished little fellow who displayed great embarrassment. Of the same general nervous appearance was 48148, but in this instance she had a perfect set of teeth. The two with factors above 80 were normal appearing, with no distinc-

tive characteristic except that one had excellent and the other poor teeth.

All nationalities were represented in our cases. Irish children abounded, with Jewish and French as close seconds. There was one colored girl with an immune mouth. These nationalities were placed in both immune and carious tables.

A glance at any of the tables displays the fact that care of the teeth played no part in either the dental condition or the salivary factor. There was sometimes doubt with both immune and carious cases as to the veracity of their acknowledgment of care.

Every sample of saliva was collected at least two hours after a meal. The character varied considerably, but not consistently. Table 7 shows this. The activated saliva of every patient was darker colored, less viscid and secreted more rapidly than the resting saliva.

TABLE 7

CASE NO.	DENTAL CONDITION	CHARACTER OF SALIVA	SALIVARY FACTOR
47084K	Decay	Evil smelling, bloody, viscous, secreted slowly	164.26
47367	Immune	Dark, viscous, secreted slowly	67.60
1905A	Decay	Colorless, clean, secreted rapidly	38.00
1908A	Decay with care	Opaque, secreted with average rapidity	49.07

SUMMARY

From observations made on these cases, it would appear that the saliva of persons with teeth immune to caries varies, as does also the saliva of persons with carious teeth; that saliva may neutralize substances taken into the mouth and that the average immune mouth has the greater power of neutralization; but the ratio of resting and activated saliva in immune mouths does not vary enough from that of carious mouths to prove that this ratio is indicative of the production and maintenance of immunity from caries in any individual.

The tables compiled do not show consistently that as the difference between the total neutralizing powers of resting and activated saliva diminishes, liability to incidence of caries increases.

Since the average ratio or "salivary factor" is below 80 in both immune and carious mouths, there is doubt as to the importance of this mark in the relation of the salivary factor and dental caries.

Furthermore, no constant points of difference can be found to correspond with the differences in salivary factors and in our work we can find no substantial proof to verify a relationship of the salivary factor to dental caries.

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THE INFLUENCE OF MUSIC UPON ELECTROCARDIOGRAMS AND BLOOD PRESSURE

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The object of the experiment of which only the beginning is here briefly summarized, is to ascertain the effect of different kinds of music upon the heart and blood pressure in individuals who are known to have musical talent and are fond of music; also in persons who are indifferent and have no fondness for music, in neurasthenics and in some animals.

The cardiograms were recorded with the Einthoven string galvanometer, its sensitivity was 1 cm. deflection per one millivolt; the film speed was 2.5 cm. per second. The time marker recorded $\frac{1}{5}$ second, and lead 2 was adopted for comparison, for all of the records.

The pulse and pressure were obtained with a Tycos and a modified form of Erlanger's sphygmomanometer, and the music from Victrola records.

The pieces of music selected were first, Tschaikowsky's death symphony, characterized by its tragic slow minor movements; and second, the Toreador's brilliant description of the bull fight from Carmen; and third, the National Emblem, a stirring rhythmical march by Sousa. The effects of other pieces of music had been tested but the results seemed to indicate that the effects of those pieces that were familiar to the subjects, were influenced also by associated memory.

The data from subject "A" who is fond of music and whose voice has been cultivated, were checked up with those of two other subjects, but his being most complete were chosen for presentation.

The experiments were conducted under fairly constant subjective and weather conditions, and about the same hours of the day. The cardiograms, pulse and pressure curves were secured before and immediately after, and also from five to ten minutes after the music had ceased. But while listening to the music, only the cardiograms were taken because it seemed that the latter were affected by the manipula-

tions necessary to secure blood pressure records. More experiments are needed before it is possible to state how long the after effects of the music persist, and also to ascertain if different kinds of quality or timbre may have different effects. The same piece of music if sung or played by the orchestra or piano or violin might have a different influence. For control data, the subjects' cardiograms, pulse and pressure were obtained, without the influence of music, at different hours of the morning, the forenoon being the time during which the tests were all made. It was found that ordinarily the pulse rate and pressure vary somewhat during the forenoon as shown in table 1.

The fact that the pulse rate decreases and the pulse pressure increases as the morning advances must therefore be taken into consideration in estimating the after effects of music.

From a study of the table, we learn that while listening to the sym-

TABLE 1

Mean results of the morning variations: in pulse and pressure

TIME	PULSE PER MINUTE	SYSTOLIC PER MILLIMETER	DIASTOLIC PER MILLIMETER	PULSE PRESSURE PER MILLIMETER
10.45	84	112	76	36
11.15	82	114	76	38
11.30	78	114	74	40
11.45	76	114	72	40
12.00	72	114	74	40

phony, the average effect is a slight decrease if any of the "P. P." wave, and therefore a relatively slight increase in the pulse rate, and also that the amplitude or E. M. F. of the "R" wave is increased. We find also, that from two to ten minutes after the music has ceased, the pulse rate and the E. M. F. have increased considerably, but the systolic and pulse pressure have fallen.

Consequently the minor tones of the symphony records caused an increase in cardiac activity and action current, but a fall in blood pressure. The increased pulse rate and decreased blood pressure are probably the result of psychic or reflex inhibition of the vagus nerve and vasomotor center. The shortening of the "P. P." wave is mainly due to the decrease of the "T. P." wave, or pause, of the cardiac cycle.

Toreador's stirring song produced a different picture of cardiograms, seen by an inspection of table 2. We find that the pulse rate was accelerated and the E. M. F. or amplitude of "R" wave became less as

soon as the song was heard. This is graphically shown in the decrease of the "P. P." and "T. P." phases, and height of the "R" wave in cardiograms obtained while listening to the song. The after effect was increased systolic and pulse pressure and pulse rate, but decreased action current. It seems, therefore, that this kind of music had a stimulating effect upon the circulation by increasing the blood pressure and pulse rate while lessening the action current of the ventricular con-

TABLE 2

Summary of cardiograms and pressure

MUSIC	CURVE	TIME	RELATIVE TO MUSIC	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE PER MINUTE	"P. P." PER CUBIC MILLIMETER	"T. P." PER CUBIC MILLIMETER	"E. M. F." AMPLITUDE "R"
				mm.	*mm.*	*mm.*				
Symphony	54	11.15	Before	110	65	45	76	1.98	0.82	0.50
	55	11.20	During				83	1.80	0.72	0*55
	56	11.25	During				75	2.00	0.85	0.56
	57	11.30	During				73	2.03	0.90	0.55
	58	11.32	After	102	60	42	80	1.86	0.74	0.58
	59	11.36	After	102			81	1.84	0.71	0.58
Toreador	46	11.00	Before	106	78	28	81	1.84	0.77	0.60
	47	11.5	During				83	1.80	0.68	0.58
	48	11.10	During				87	1.71	0.58	0.56
	49	11.15	After	112	74	37	85	1.76	0.65	0.57
National March	41	11.40	Before	106	70	36	75	2.00	0.80	0.60
	42	11.50	During				70	2.14	0.90	0.62
	44	11.57	During				71	2.09	0.85	0.63
	45	12.00	After	112	66	56	75	2.10	0.84	0.70

1 cm. per millivolt. film speed 2.5 cm. per second. Time $\frac{1}{5}$ second. Lead 2.0 for all records.

traction. This change may be due to reflex action of the accelerator nerve or possibly inhibition of the vagus.

The effect of the inspiring rhythmical tones of the National Emblem, as seen from table 2, was a slower pulse rate, a longer pause or "T. P." wave, and an increase of not only the systolic and pulse pressure but also the action current of the ventricular contraction. It seems that this music had its stimulating effect upon the vagus, and that this as

well as other kinds of music may have an influence on the system in other respects. It very likely affects digestion, secretion, muscle tone, and respiration. But many more experiments are needed before definite conclusions can be drawn. A survey of the preliminary results obtained with the three classes of music indicates that in the subjects experimented on, the minor tones of music increased the pulse rate and action current of the ventricular contraction, and lowered the systolic and diastolic pressures. On the other hand, the stirring notes of Toreador's song, and also those of the rhythmical march, increased the systolic and pulse pressure, but the former also increased the pulse rate, with decreased diastolic pressure and action current, while the march slowed the cardiac cycle and increased its action currents. It is possible that a careful selection of music may be a beneficial aid in the treatment of nervous disturbances.

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